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THE RICKETS-PRODUCING EFFECT OF BERYLLIUM CARBONATE*
[L'azione rachitogena del carbonato di berillio]

By

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Summary

Some experiments carried out with young rats have indicated that beryllium carbonate can adversely affect the osteogenic processes leading to a series of pathological manifestations including weight loss, curving of the spine, hyperplastic alterations of the conjugation cartilage, modifications of the underlying trabecular structure, hypocalcification, thickening of the chondriocostal joints.

This rickets cannot be attributed to a simple deficiency, since it can be brought about also through maternal milk, and since it cannot be modified by cod liver oil or by vitamin-containing preparations; a broader pathogenic process must be involved, in which toxicity may have the main role.

Studies in the field of experimental pathology are obviously and closely related to occupational diseases. This is not only due to the information which can be provided by experiments relating to certain points

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*From the Institute of General Pathology of the University of Rome (Prof. Guido Vernoni, Director). This work received an award at the first "Ferdinando Micheli Prizes" contest, sponsored by the E.N.P.I.

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of an ill-defined syndrome, but also to the protection that may derive for the worker.

The work is usually carried out before its hygiene is established. It is sufficient to remember what happened with the best known poisonings. The worker came into contact with lead, mercury, phosphorus, antimony, benzene, etc., suffered their various pathogenic effects, and finally, only when the morbid symptoms reached substantial proportions as to frequency and intensity, could the worker receive due prophylactic precautions. Thus these, in the great majority of cases, derive directly from the "a posteriori" finding of a pathogenic relationship between work and worker.

At the present time, especially because of the remarkable development of industrial activities and of the constantly progressing assistence to the working classes, labor hygiene has been steadily improving. It should, however, be emphasized that if there were a tighter connection between labor and experimental pathology, the prophylactic measures in favor of the workers would be much improved, especially as far as timeliness is concerned.

Modern industry, due to the effect of new demands and new discoveries, every year acquires new forms of activities. These are new techniques, new physical and chemical processes, involving directly large numbers of workers, very often before it has been possible to detect in the new work the existence of elements which may at some time become pathogenic factors. Thus, experimental pathology should be in the foreground, together with the progress of technical findings. By a timely evaluation of the new elements of the industrial activities in the more important physio-pathological aspects, it could provide valuable indications which might eventually avoid a useless experimental confirmation in man. It should be kept in mind that in this

field, as in many other fields of medicine, today's experimental detail may constitute tomorrow's practical finding.

Beryllium

Beryllium is a metal which in recent times has been acquiring an increasing importance in the field of metallurgical industry. Very recent experimental studies, among which should be remembered those by Losana, have shown that this metal increases the hardness and elasticity of other metals, light and heavy, when it is combined with them as an alloy. This is very useful in the manufacture of engines, especially in the aeronautics industry, where lightness must be combined with resistence.

In view of the considerable difficulty in obtaining it in a pure state, many of its properties have been determined in recent years, among others through important studies performed at the Institute of General and Applied Chemistry of the Turin Institute of Technology. Its atomic weight is 9.02, melting point 1285°C, specific gravity 1.84, hardness, rather high, between 6 and 7 on the Mohs scale, and 150 Brinell; its modulus of elasticity is about 30,000 kg, thus very high. Its symbol is Be. It belongs to the alkaline earth metals group, is bivalent, and its properties are similar to those of aluminum and magnesium. When pure it is silvery white. It combines very easily with halogens; when heated it reacts with oxygen and nitrogen, and at high temperatures it reacts with carbon and boron.

It was isolated for the first time by Wohler and Bussy in 1828, and after the initial studies, when it was found that its salts had a sweet taste, it was also called "glucinium." In nature it is found in various combinations,

together with other compounds, such as aluminum silicate, aluminum oxide, etc. The most important mines are those in Madagascar, India, South Africa, Portugal, etc. In Italy there are some mines in the Alto Adige region, in Lombardy and, according to recent information, there are mines in Ethiopia.

The methods of preparation are very complicated and expensive and, because of their difficulty, they are kept secret in the various factories. The Siemens Halske process begins with the disintegration of the beryllium-containing material, together with an equivalent weight of sodium fluorosilicate at 650-700°C. After digestion of the melted material with cold water, beryllium hydroxide is precipitated from the double fluoride of beryllium and sodium by addition of calcium hydroxide. Subsequent treatments with hydrogen fluoride permit obtaining pure oxyfluoride. Another procedure involves disintegration of the material by melting with potassium carbonate. After several treatments beryllium hydroxide is obtained, from which salts are obtained by reaction with acids. The pure metal is obtained electrolytically in good yield. Electrolysis is carried out with graphite electrodes at 1350°C using a melted mixture of beryllium fluoride and barium fluoride.

Among its compounds the following should be mentioned: the oxide, which when irradiated with alpha particles coming from radium disintegrates with expulsion of hydrogen ions; the chloride, colorless, which melts and volatilizes easily; the carbide, the hydroxide, the sulfate, the silicate, the carbonate, etc.

Until recently the difficulty of the methods of preparation caused a high price of beryllium: about 5000 lire per kg. A new procedure developed by Carlo Adamoli and his coworkers in Italy has in part overcome this

difficulty. The process is essentially as follows: preliminary heating at high temperature with sodium hydroxide, which gives beryllium oxide. After other treatments this is transformed, in large vacuum furnaces, into a beryllium halide. Instead of being treated electrolytically, the salt is then melted together with catalysts, in special electric furnaces, and is made to react with different alloys according to what product is desired. This process permits production of ingots of alloys containing up to 30% beryllium, a very high percentage, never before obtained.

The most interesting property from an industrial viewpoint is, as mentioned before, the ability to give alloys of great hardness and resistance. For instance, when copper is combined with beryllium in an alloy containing only 2% of the latter, after appropriate heatings, it is possible to increase tenfold the breaking load and the hardness, while at the same time decreasing its ability of elongation. Considerable increases are also obtained with nickel in an alloy containing 1.7% beryllium. Aluminum and magnesium are also improved by formation of an alloy with beryllium; this will permit wider uses of light alloys. When iron is combined with beryllium the hardness and certain magnetic properties are increased; these properties are still being studied in Italian technical laboratories.

Physiopathological Effects of Beryllium Carbonate

Menesini, in a study on beryllium carbonate and its biological effect, has observed that very little work has been done on the physiopathological effects of this metal. The first work was done by Siem, who in 1886 found that subcutaneous injection of beryllium caused the occurrence of hemorrhages, gastroenteritis, nephritis and paralysis. Still in the field of pathology,

Comar has studied the effect of intravenous injection of various beryllium compounds in the rabbit. He reported alterations of the liver, spleen and adrenals, with strong reactions of the reticulo-endothelial system. Lunde, Gessner, Siebert and Helms claim to have favorably influenced the course of human and experimental tuberculosis by intravenous treatment with beryllium salts. Cuneo reports good results obtained by parenteral treatment with beryllium chloride in cases of tuberculosis, staphylococcal infections, and tumors; he detected an antitoxic effect, and a stimulation of hemopoiesis and of metabolism. Fabroni, who did much research work on beryllium, obtained an improvement of the weight increase of guinea pigs treated with small doses of beryllium chloride, iodide and hydroxide. He also observed an antitoxic effect of beryllium hydroxide against lead acetate and arsenious acid. The same author elicited bronchopneumonia in guinea pigs by inhalation of beryllium carbonate. Menesini obtained similar results, but found an inability to determine histogenetic processes. He considers it a very harmful substance.

Gelmann's observations are more directly related to labor pathology. He reported that the vapors evolved during melting of beryllium-containing materials cause initially an increase of temperature in the workers, followed by dyspnea, cough, cyanosis, indicating lung and bronchial involvement. Gelmann attributes this syndrome to beryllium oxyfluoride vapors.

Beryllium Carbonate and Experimental Rickets

The work on experimental rickets is mentioned last because it is more directly concerned with my own work.

Studies on rickets have undergone a wide development since the first classical observations of Glisson. The essential pathogenetic cause was found to be a decrease of inorganic P in circulating blood, which caused a

change in the Ca/P ratio. The reason for this was found to be either a decreased entry of phosphorus or the inability, in varying degrees, of the body to liberate inorganic phosphorus from organic phosphorus compounds. The presence of vitamin D is essential for the regular functioning of this metabolism. Today however many alterations of osteogenic processes of the rickets type are due to toxic or infectious effects which are not readily explained with a too simple avitaminosis criterion; this has been shown by Rezzesi in a broad and well-documented monograph on this subject. Clinicians such as Frontali and Marfan, just to mention a few, had already observed the complexity of rickets pathogenesis.

This has been brought out more decisively in the experimental field. It has been observed that, in addition to the various rickets-producing diets (Bacharac), many other substances are able to exert a pathogenic effect on the processes of bone growth, and also to cause clear rickets cases. Lowe, Steenbok, Bruin, and Bouman obtained this by using cereals; Sobel, Cohen, Kramer, Shipley, Park, MacCallum, Simmond, and recently Roche and Mourgue, by adding strontium to normal diets; Buschke and Pieser using thallium. Other substances also have this property, and many of them have been found to be resistant to vitamin treatment and to ultraviolet irradiation. A strong rickets-producing action on the bones of young growing rats was reported for beryllium carbonate, for the first time, a few years ago, by the Americans Guyatt, Kay and Branion. They added to the normal daily diet beryllium carbonate in amounts from 0.125% to 2% of the weight of the food, and succeeded in causing in young rats the appearance of rickets, which was confirmed also by histological examination. Fabroni, however, continued these experiments and found no changes by treating young guinea

pigs with 1% and 2% beryllium carbonate; he therefore maintains that the alterations found by the American authors must essentially be attributed to fluoride impurities.

Effect of fluorine. Before reporting the experiments, it is appropriate to discuss the effect of fluorine suggested by Fabroni as the cause of the alterations observed by Guy, Kay and Branion.

It can be stated immediately that experiments carried out with fluorine or its compounds have led to the observation of various biological effects; the bone lesions however are mostly modifications of the osteoporotic type (Rost, Cristiani, Marconi, Slaviero, etc.); some authors consider them to be similar to rickets, but the majority of the authors consider them sharply different from rickets. Mascherpa and Lusignani (1936) observed that chronic poisoning with sodium fluoride causes variations of the calcium content in guinea pig bones; these variations are kept within moderate limits if vitamin D is administered simultaneously. Work by Cannava has confirmed this observation, and added new information on the pathogenetic details. Bardelli and Menzani, while studying a serious industrial fluorosis that occurred in a Venetian valley, observed in the poisoned animals a progressive decay of the physical conditions, and in the bones at first regression, and later hyperplastic alterations. Histological examination showed in some sites impoverishment of the bone tissue, and in other sites a strong hyperplasia of the connective tissue adding up to an overall picture very similar to that usually described as fibrous osteodystrophy, or Recklinghausen disease. Experiments by Gettler and Ellebrook, published in 1939, report as the main findings in chronic fluorine poisoning organic decay and mobility disturbances. Finally, in the specific case of rickets, the experiments of McCollum,

Simmond, Becker and Rezzesi have ruled out the possibility that fluorine may cause typically rachitic lesions.

In conclusion, based on the studies mentioned, it does not appear that the rachitic alterations obtained by Guyatt, Kay and Branion can be attributed to the presence of fluoride impurities.

Foreword to Experimental Section

The matter of a possible rickets-producing dysosteogenic effect of beryllium carbonate was still experimentally open, in view of the conflicting results reported by the American authors and by Fabroni. On the other hand, it was useful to study the effect of impurities (fluorides, chlorides, etc.) on the possible biological effect of beryllium carbonate. I therefore decided to continue the experiments, with some changes, using beryllium carbonate obtained from Landau, a company that always offered very pure laboratory chemicals. As a comparison, I have also used, in other experiments, beryllium carbonate obtained from the Riedel Company, which in preliminary experiments had appeared less pure than the other one. With these experiments I have attempted also to clarify some biological properties of a compound that, as I mentioned above, is becoming more and more important in modern industry. Many plants for the large scale production of beryllium have been built recently, since the finding of the ability of beryllium to form highly resistant alloys. The best known foreign plants are those of Siemens and Halske in Berlin, the Heraeus Vacuum Schmelz of Hanau, the Alais Froges and Camargue in Paris, the Beryllium Development Co. of New York, and British Metals of London.

In Italy studies on the utilization of beryllium alloys began about 10 years ago. The S.A. Processi Privative Industriali, which kindly provided me with many data, began its activity in 1931 in Milan, and continued it on an experimental and semi-industrial basis until 1936. Having obtained successful results during this period, the company started a plant in Chivasso (Turin) which has been active since 1937. It produces beryllium oxide, beryllium fluoride, metallic beryllium and its alloys. The manufacturing process has been patented. Ammonium difluoride and sodium difluoride are used in this process, and vapors are evolved and eliminated in a closed system. About 150 workers are currently employed in this plant. The importance of this type of production permits anticipating a fast development of this new industrial activity which has been so well established also in Italy.

Growing rats about 30 days old, weighing about 30 grams, were used in the experiments. Each lot of rats was divided in two groups, kept under identical conditions; group A was administered beryllium carbonate, and group B was the control. Beryllium carbonate was mixed in the morning with fresh milk. As the rats grew older, the diet was modified to include soup, bread, vegetables, etc. Beryllium carbonate was administered also to pregnant rats, and its effect on the offspring observed, in order to detect any effects on intrauterine life. I have also studied the possible effect of cod liver oil and of vitamin preparations on the alterations brought about by beryllium carbonate. In order to handle the data on the effects of beryllium carbonate on the rats whose mother had this compound administered to her, I determined the average weight of normal rats in our litters in the first days of life. The results are as follows:

First day:	4.7 g
2nd	5
3rd	5.7
4th	6.3
5th	7
6th	7.5
7th	8
8th	8.3
9th	8.5
10th	9.3
12th	10.
13th	10.5

We examined the aspect of the rats during treatment, checked their weight, and carried out radiological and histological examination of the rats which died or were sacrificed when they were in poor physical condition. The same studies were performed on the control animals.

In order to bring about experimental rickets, the American authors administered to the rats daily amounts between 0.12% and 3% of the weight of the daily diet. In the experiments reported here, we have determined the following averages:

Age of Rat	Average Weight of Rat	Weight of Meal
I 30 days	30 g	6 g
II 35 days	35 g	7 g
III 40 days	40 g	8 g

from which the following percentages of beryllium carbonate are calculated:

	0.25%	0.5%	1%	2%
I	0.015 g	0.03 g	0.06 g	0.12 g
II	0.0175 g	0.035 g	0.07 g	0.14 g
III	0.02 g	0.04 g	0.08 g	0.16 g

For each 5 g of weight of the rat over 40 g, the following amounts are added:

0.25%	0.5%	1%	2%
0.0025 g	0.005 g	0.01 g	0.02 g

In the experiments reported here we have used the above amounts as tentative figures. In certain instances we have been obliged to vary the amounts of beryllium carbonate in order to allow the animals to survive.

For the sake of brevity, we shall not report the experimental protocols.

Experimental Details

Experiment No. 1. The rats of this experiment were divided in two groups. Group A was treated daily with 0.150 g of Riedel beryllium carbonate per animal; group A' received 0.075 g. All the rats of group A died after 3 days, those of group A' after 6 to 9 days. In this case the death of the rats was most likely caused by the high doses of Riedel beryllium carbonate.

Experiment No. 2. The rats of this experiment were divided in two groups. Group A was treated daily with 0.06 g of Riedel beryllium carbonate per animal. Group B was the control. After a few days the rats of group A were less lively. On the 5th day of the experiment two rats of group A died; they weighed an average of 5 g less than the control group. The poor condition of the rats in the test group suggests that administration of beryllium carbonate should be discontinued. Four drops of cod liver oil were added to the diet. After 5 days, group A was in good condition, and its weight was the same as that of the control group. Administration of beryllium carbonate was resumed, at a reduced dose of 0.015 g per rat. Seven days later another rat of group A died; it weighed 20 g less than the corresponding control. Fifteen days after the second administration of beryllium carbonate, rat No. 4 of group A died; it weighed 40 g, while the control rats had an average weight of 73 g.

Experiment No. 3. The rats of this experiment were divided in two groups; group A was given 0.037 g of Riedel beryllium carbonate per animal.

Group B was the control. After 3 days several rats of groups A and B died, most likely for reasons independent from the experiment. In view of the poor condition of the rats, the administration of beryllium carbonate was suspended for 4 days, and 3 drops of cod liver oil were added to the daily diet of group A and of group B. Administration of beryllium carbonate at a reduced dose was resumed (0.025 g per animal), but after 7 days three rats of group A died; their average weight was 32 g, compared with 40 g of the control group. Administration of beryllium carbonate was temporarily discontinued, and cod liver oil was again administered. After a 13-day interruption, during which one more rat died, administration of beryllium carbonate at a reduced dose of 0.0075 g was resumed. Ten days later, the 7th rat of group A, and finally on the 45th day after the beginning of the experiment, also the 8th rat of that group died.

For the whole duration of the experiment the rats receiving beryllium carbonate were less lively and had a sharply curved back, due to a definite hump. This curving of the spine was not due to the animals' attitude, but to an anatomical alteration, which prevented even its passive straightening.

Experiment No. 4. This experiment had the purpose of checking whether the effect of Riedel beryllium carbonate administration to a pregnant rat may have an effect on the offspring. For this purpose a female rat weighing 175 g received 0.075 g daily. The dose was immediately reduced to 0.037 g, because the rat did not appear to tolerate beryllium carbonate. The rat delivered after 3 days, and its three offspring weihed 5 g on the average. Administration of beryllium carbonate was continued, and it was observed that the mother ate little and that the nursing was not satisfactory.

Twelve days after birth, the rats did not have a good appearance. They barely moved, were in a curved position, and their spine was strongly curved. They were killed; their weight was 8 g, as compared with 10.5 g for normal rats of the same age.

Experiment No. 5. In this experiment we used beryllium carbonate from the Landau Company.

The rats were divided in two groups. Group A received 0.06 g of Landau beryllium carbonate per animal. Group B was the control. After 14 days, since it was found that the two groups had the same weight and that group A did not appear to suffer from beryllium carbonate administration, the daily dose was increased to 0.16 g. Only after about 10 days was a weight difference observed. After 19 days, the dose of beryllium carbonate was further increased to 0.24 g per animal. This dose further slowed the weight increase of the rats [of group A] which weighed about 10 g less than the controls. Forty days after beginning the experiment, the average weight of the rats of group A was 68.5 g, while the control rats weighed 87 g.

At this point the rats of group A were divided in two groups. Group A_1 received 0.24 g of beryllium carbonate with the addition of two drops of Vigantolo. Group A_2 received the same dose of beryllium carbonate without additions. After about 30 days the animals receiving Vigantolo weighed a few grams more than those of group A. Both groups weighed about one half as much as the control animals (130 g).

The rats receiving Landau beryllium carbonate lacked liveliness, their development was reduced, and they had humps.

Experiment No. 6. A pregnant rat received Landau beryllium carbonate at a dose of 250 mg/day. After 4 days the rat delivered four offspring, which died immediately.

Experiment No. 7. A pregnant rat received Landau beryllium carbonate at a dose of 0.5 g/day. The rat delivered after 3 days but the four offspring died the same day.

Experiment No. 8. A pregnant rat received Landau beryllium carbonate at a dose of 0.5 g/day. The rat delivered after 2 days, but the offspring died the same day.

Radiographic Examinations

Radiographic examinations were carried out on some rats belonging to the test and control groups, in order to establish whether there were any alterations of the morphology and the mineral content of bones. Based on observations by other scientists, we studied with special care the development of single bones, their thickness and the calcification of the different sections, especially those involved in osteogenesis, the possible enlargement of the ossification areas, especially the knee joint, the morphological behavior of the chondriocostal joints and of the spine.

Experiment No. 2. Radiological examination of rat No. 4A was carried out. This rat had died on day 14 of the experiment in which the animals received 0.015 g of Riedel beryllium carbonate. Its weight was 40 g, while control 2B, which was also x-rayed, weighed 70 g. Radiographic examination of rat 4A, compared with the control, did not show any appreciable morphological or calcification alterations. The outline of the long bones is perfectly normal both in the diaphysis and epiphysis. Calcification in

these bones is also normal. The only clear alteration is a much smaller development of the long bones of rat 4A relative to rat 2B.

This finding is in agreement with the weight difference of these two animals, reported above. It should also be noted that the shadows of the chondriocostal joints are much sharper in rat 4A than in rat 2B.

Experiment No. 3. Radiographic examination of rat 4A was carried out. This rat weighed 21 g; it died on the 14th day of the experiment, in which 0.025 g of Riedel beryllium carbonate had been administered. Rat 5A, weighing 27 g, which had undergone the same treatment, was also examined. In addition, rat 5B of the control group was also examined radiologically.

The long bones of rat 4A had normal shape and calcification. The ossification processes in the femural distal epiphysis appeared to be slowed down. The same ossification processes at the head of the humerus were also delayed. The long bones were smaller than in the control animals, thus their growth ability was decreased. A sizeable curving of the spine was also observed, especially in the lower third of the dorsal region.

The same changes were observed in rat 5A, especially the decreased development of the long bones and the curving of the spine in the region described. No alteration of the morphology and calcification of bones was found here either. No other significant finding was made.

On the 37th day of experimentation, radiographic examination of rat 7A was carried out; this rat weighed 36 g and was receiving a reduced dose of 0.0075 g of Riedel beryllium carbonate. The same examination was done on rat 6B, which weighed 68 g and belonged to the control group. The long bones of rat 7A were less well developed than the controls; a slightly decreased calcification was found. The head of the tibia was considerably

thickened in the area of the epiphyseal cartilage. A similar alteration was observed at the head of the humerus. Lateral examination of rat 7A showed a very considerable curvature of the spine, due to a stronger emphasis of the normal curves with posterior concavity in the cervical region, and of the curves with anterior concavity in the dorsal region. As we said above, this abnormal attitude taking place gradually is obviously due to a morphological alteration, since it could not be straightened out even immediately after death; we therefore examined the outline of the spine. Comparison of the pictures from rats 7A and 6B did not show any particular morphological alteration.

On day 43 of the experiment, radiological examination of rat 8A was carried out. This rat weighed 50 g, and was receiving the reduced dose of 0.0075 g of Riedel beryllium carbonate. The same examination was done on rat 7B, weighing 85 g, belonging to the control group. Radiography of rat 8A shows scant development and calcification of long bones, compared with the control. The relationship between bone and cartilage in the femur-tibia joint is also unusual. The spine of rat 8A was also found to have a sharp accentuation of the cervical and dorsal curves.

Experiment No. 4. Radiographic examination of rats 1A and 2A, offspring of a rat receiving 0.037 g of Riedel beryllium carbonate for the last 3 days of pregnancy and through the nursing period was carried out. Rats 1 and 2A, sacrificed on the 15th day of the experiment, weighed 8 g each, while control rat 1B, which was also radiographed, weighed 11 g. Rats 1A and 2A had a delayed development of the long bones and a decreased ossification due to inadequate proliferation, especially in the epiphyseal regions. Observation of rats in a lateral position allowed the observation to be made that curving of the spine takes place early.

Experiment No. 5. Radiograms were taken of rat 1A weighing 50 g. This rat was sacrificed on the 48th day of the experiment, and had been receiving 0.24 g of Landau beryllium cabonate. A radiogram was taken also of control rat 2B, weighing 87 g.

The long bones of rat 1A have normal structure and calcification, although they are less well developed than the control. The head of the tibia shows a sharp separation of the metaphysis; this indicates a considerable thickening of the epiphyseal cartilage. Development and calcification of the distal epiphysis of the femur, and of the proximal one of the humerus, were decreased. Upon lateral examination, rat 1A showed a sizeable curvature of the spine in the thoracic and cervical regions. Radiography showed a decreased calcification of the vertebrae, which might explain the origin of the curving.

On the 77th day of the experiment, rat $1A_1$ was sacrificed; it had been receiving 0.24 g of Landau beryllium carbonate, and for the last 27 days also 2 drops of Vigantolo. It weighed 62 g. Radiograms of this rat and of control rat 3B, weighing 122 g, were taken. The long bones of rat $1A_1$ were of normal configuration and calcification, but their development was decreased relative to the controls. At the head of the tibia and at that of the humerus there was an increased development of the epiphyseal cartilage. The spine of rat $1A_1$ was also quite curved in the cervical and dorsal regions, and the spine itself was less calcified than that of the controls.

On the 83rd day of the experiment rat 2A was sacrificed. It had been receiving 0.24 g of Landau beryllium carbonate and weighed 58 g. Rat $2A_1$, weighing 60 g, was also sacrificed. It had been receiving 2 drops of Vigantolo for the last 33 days, in addition to beryllium carbonate. Radiograms were taken of these rats and of rat 4B, weighing 130 g. The long bones

of rat 2A were less developed than those of the control. The epiphyseal zones of the tibia-femural joint were irregularly calcified. The development of the proximal epiphysis of the femur was decreased, and calcification was also decreased. The spine had the two curves already mentioned several times; they were not very marked. This rat had a very strong hyperplastic alteration at the level of the chondriocostal joints. This is identified as the classical rachitic rosary.

The radiogram of rat $2A_1$ showed a considerable thickening of the femur with sizeable calcification irregularities at the distal epiphyseal area. These irregularities were found also at the head of the femur and of the humerus, where an increase of the epiphyseal cartilage was found. A radiogram of this rat in sagittal position showed a hint of the rachitic rosary. The curves of the spine were moderate.

Histological Examinations

A regular histological examination of the bones of test and control rats was performed, with special attention to the areas involved in growth processes.

The most marked alterations were observed in the epiphyseal heads of the tibia-femural joint (especially in the proximal epiphysis of the tibia), at the head of the humerus, and in the chondriocostal joints.

A description of some of the more typical histological lesions is given.

Experiment No. 5. Rat 1A treated with Landau beryllium carbonate for 48 days (0.06 g - 0.16 g - 0.24 g).

Tibia. Examination of the proximal epiphysis showed that the conjugation cartilage had lost the normal sharply outlined linear structure of

the underlying bone layer. Especially in its median part, it was bordered with thick cell shoots plunging in a disorderly fashion into the underlying tissue. A detailed examination of the various parts of the cartilage showed that there was a tendency to hyperproliferation in the cells of the hyaline layer. This became stronger in the serial cartilage, in the area of globular cells, where, instead of layers of 5-7 cells, thicker layers of 40-60 cells were observed.

The underlying tissue did not separate from the cartilage through thin and distinct bone trabeculae outlining wide marrow spaces rich in cells. Instead, it was abnormally dense with rough, poorly calcified, cell-poor trabeculae, and interrupted by short marrow gaps where marrow cells and osteoblasts were decreased in number. The structural disorder is also evidenced by the persistence of cartilaginous islands of various sizes, within the osteoid tissue.

Vertebrae. A sizeable increase of the serial cartilage was noted in the vertebral bodies, expecially in the layer of inflated cells (18-20). The ground substance is poorly calcified. Below the cartilage, instead of the bone trabeculae and vascular ansae, there are wide masses of compact osteoid tissue, of irregular calcification, interrupted here and there by blood-rich marrow gaps. It should also be noted that the central part of the vertebral body is almost totally occupied by a mass of marrow substance, while normally it should have its regular calcification.

The vertebrae of the most curved area have, in addition to the qualities just described, a plastic deformation of the cortical layers which took place due to incomplete calcification of the bone periphery.

Experiment No. 3. Rat 5A, treated for 14 days with Riedel beryllium carbonate, in daily amounts of 0.037 g - 0.025 g.

Tibia. The conjugation cartilage is abnormally thickened especially in the median area. This increase in thickness takes place at the expense of the inflated cells of the serial cartilage (18-25). The border with the ossification area is very irregular and the persistence of islets of cartilage tissue is observed in the bone tissue. A clear trabecular structure is absent; this becomes clearer lower down in the parts near the diaphysis. The calcification processes appear to be fairly regular.

Fibula. The same basic findings as in the corresponding parts of the tibia are observed in the proximal epiphysis. This is an abnormal proliferation of the serial cartilage, disappearance of the axial structure of the bone trabeculae and their replacement with a more compact tissue, interrupted in a disorderly fashion by marrow gaps.

Vertebrae. The serial cartilage in some of the vertebral bodies is clearly hyperplastic. Below the cartilage layer there is a considerable deficiency of osseous trabeculae, with an anomalous abundance of medullar tissue in their place. The most curved parts have a decreased calcification. A reduced development of the intervertebral disks was observed.

Chondriocostal joints. The serial cartilate is the most changed, with increase of globular cells (18-25) and loss of the trabecular structure in the underlying bone tissue.

Experiment No. 2. Rat 4A treated with Riedel beryllium carbonate for 26 days. Daily dose: 0.015 g.

Tibia. The conjugation cartilage has the typical increase in thickness in the median zone due to hyperproliferation leading to the formation of long shoots of globular cells entering irregularly into the underlying tissue. In its border area this is irregularly calcified, while the trabecular structure, which can be fairly well recognized, is variously fragmented. Thickening of the osteoid tissue to a compact mass was not observed here, while it was observed in rats having received Landau beryllium carbonate.

Experiment No. 2. Rat 3A treated with Riedel beryllium carbonate for 11 days. Daily amount: 0.015 g.

Tibia. The epiphyseal cartilage shows the usual alterations in the serial zone, with sizeable hyperplasia of the globular cells (40-50). It should be noted that the ossification zone has its normal trabecular structure.

Summary of Results

Riedel and Landau beryllium carbonate. Based on the experiments reported it was found that beryllium carbonate causes rickets of varying severity, depending on whether the compound was prepared by the Riedel Company or the Landau Company. The Riedel product, in addition to rickets-producing activity, had a fairly strong toxicity, causing in some experiments a rapid decay and often the death of the animals.

The rats of group A in Experiment No. 1, which received 0.150 g of Riedel beryllium carbonate each, all died within 3 days, and those of group B, receiving 0.075 g of the same compound, died within 6-9 days. A first group of Experiment No. 2 receiving a lower dose of 0.06 g died on

the fifth day, while the others died within about 20 days. The dose was reduced in Experiment No. 3 from 0.037 g to 0.0075 g, increasing the survival time to about 40 days.

The rats were much more resistant to the administration of Landau beryllium carbonate. In Experiment No. 5 it was necessary to increase the dose from 0.06 g to 0.16 g.in order to observe an effect of beryllium carbonate. The effect became more clear at a dose of 0.24 g. With this dose the weight differences between test and control rats increased. Differences were of course found also in the mobility, attitude, etc., without any deaths.

Looking at the amounts needed to cause rickets, it is found that the Riedel beryllium carbonate is more active, at lower doses, than the Landau product. The beryllium carbonate from Riedel was poorly tolerated by the rats; this may suggest that the presence of impurities (fluorides, chlorides, etc.) may be responsible for the general toxicity and for a special pathogenic effect on the bone tissue which, weakened by these impurities, is more sensitive to the rickets-producing effect of beryllium carbonate. This is supported by the lesions caused by fluorine on the bones. On the other hand, the fact that the Landau beryllium carbonate, a product which is guaranteed to be absolutely pure, caused typically rachitic lesions, permits attributing to this compound the ability to cause the experimental rickets obtained in the rats.

Beryllium carbonate and its effect on rats. The effect of beryllium carbonate begins with a modification of the rats' normal mobility.

After a period varying with the doses, but usually between 5-8 days, they become quiet, usually stay motionless in a corner of the cage, and make only belated attempts to avoid being caught by so[illegible]ne's hand. Soon

their appetite decreases, the daily meal is not eaten completely, they lose weight and there are weight differences between test and control groups.

When this impoverished state becomes advanced, the animals curl up. The rat that had been motionless in the cage for 20 days now has a strongly bent back, especially in the dorsal region, with a curve strongly concave on the ventral side. This is not simply a position. As mentioned above, if the rat is sacrificed, it is not possible to straighten the curve by traction. This shows that a permanent morphological modification of the spine has taken place. When the rat is in this state, it reacts weakly to external stimulations. Palpation of the chest sometimes permits finding the signs of the rachitic rosary.

I mentioned that beryllium carbonate has a strong effect on weight. Differences in this effect are found between Riedel and Landau beryllium carbonate. With the Riedel product, the weight differences between test and control groups come about more rapidly and become larger in a shorter time; this is due to the synergistic effect of beryllium carbonate with the impurities present. In Experiment No. 2 with Riedel beryllium carbonate, there was a weight difference of 5 g between the average weights in the two groups of rats (test rats 30 g, control rats 35 g). On day 26, in Experiment No. 2, the weight difference reached 33 g (40 g - 73 g). In Experiment No. 3, already on day 7 the difference of average weight in the two groups was 8 g (control animals 30 g). On day 43, the weight difference between these rats was 35 g (50 - 85 g).

The Landau beryllium carbonate also had an effect on weight, but a smaller one. In Experiment No. 5, a difference of about 10 g was found only on day 10 between test and control animals (46.8 -- 56.4). Toward the end of the experiment, after 83 days, the difference of average weight

of the two groups was 70 g (60 g - 130 g). It should be kept in mind that the rats receiving Landau beryllium carbonate never appeared to be suffering, even when the experiment was nearing the end, and the weight difference between test and control animals was high.

Beryllium carbonate, pregnancy and nursing. As mentioned above, in this work I established a new experimental condition, by administering beryllium carbonate to a pregnant rat and continuing administration also during the nursing period. In general beryllium carbonate was not well tolerated. In three experiments (6, 7, 8), administration of 0.250-0.500 g of Landau beryllium carbonate was followed by the death of the offspring a few hours after delivery. Only in Experiment No. 4 with very low doses of Riedel beryllium carbonate (0.075 - 0.037 g) was it possible to keep the offspring alive. The newborn rats had normal weight, and their growth in the first 8 days was normal. After this time there was an increasing weight difference.

The radiological behavior is in agreement with this organic decay. The bones are less calcified than those of the controls, and the epiphyseal development is also delayed. Observation of the spine shows a beginning of the accentuation of the curves already described.

These experiments show that high doses of beryllium carbonate have an adverse effect on the fetus, through action on the mother and probably also directly on the fetus. With low doses no effect is found on the fetus, but in the long run there is an effect on the offspring through the milk.

Radiographic findings. The most marked alterations are found on the long bones, the spine and the chondriocostal joints.

The normal morphology is retained in the long bones. The outline of the diaphysis shows no changes, although the development of the bones is decreased as compared to the controls, and thus the bones are smaller. The sharpest changes are found in the epiphysis of the tibial-femural joint and in the head of the humerus. In the tibia there is usually a sharp increase in distance between the epiphysis proper and the metaphysis, due to a sizeable thickening of the epiphyseal cartilage. In the distal femural epiphysis there is usually a reduced and irregular calcification. On the head of the humerus there is also an increase of the space of the epiphyseal cartilage and appearance of irregular shadows showing an alteration of the calcification processes.

Due to these alterations the normal development of the epiphysis is delayed as compared to the controls.

Usually no special alterations were found in the spine. Only in Experiment No. 5 was it possible to make any observations. The vertebral bodies of rat 1A are much less calcified than those of the control. The rachitic rosary of the chondriocostal joints was demonstrated only in two cases: in rat 4A of Experiment No. 2, and in rat 2A of Experiment No. 5, much more clearly. In view of the difficulty of the radiographic observations, it is understandable that this alteration was not observed in all cases.

Calcification of the various bones was not much decreased in the test rats.

Radiographic observation of the spine permitted establishing accurately the fact that the curve in the test rats is caused by a sharper accentuation of the normal dorsally concave curves in the cervical region, and of the normal ventrally concave curves in the dorsal region.

These observations are a new contribution to the radiographic appearance of bone alterations by beryllium carbonate. The American authors Guyatt, Kay and Branion, in their report on radiographic observations, reported only a very strong decalcification of the bones of the test animals, without mentioning the other varieties of lesions that I mentioned.

Histological findings. A systematic histological examination has shown that the main rachitic alterations take place in the knee joint and especially in the proximal epiphysis of the tibia, in the head of the humerus, in the vertebrae and in the chondriocostal joints. It has been possible to observe anomalies of varying severity also in other bones.

Beryllium carbonate can cause the typical histological manifestations of rickets only after prolonged administration (about 40 days). These could be determined using the Landau beryllium carbonate which, being better tolerated, could be administered for longer periods.

The most severe alterations on the head of the tibia take place in the conjugation cartilage. Normally this has the appearance of a linear ribbon, sharply outlined and slightly thickened in the median area, on the side toward the diaphysis. The hyaline cartilage cells are continued on the lower end by those of the serial cartilage, which is followed by the area of swollen cells, usually consisting of 4-5 layers of cells. Below the cell columns are the bone trabeculae alternating with the vascular cords; they all follow an orderly axial course.

Beryllium carbonate causes, in the conjugation cartilage, cell hyperproliferation processes which histologically appear as a sizeable thickening of the serial cartilage, especially in the area of the globular cells, where up to 50-60 layers can be counted. These shoots of proliferated cartilage

plunge in a disorderly fashion into the underlying tissue which has lost its orderly trabecular structure and has the appearance of poorly calcified dense masses, variously interrupted by gaps in which there is a shortage of marrow and osteoblasts.

This picture is in agreement with the typical rachitic lesions, and indicates that there is a sizeable alteration of the normal osteogenic processes.

When beryllium carbonate acts for a shorter time, the same increase of globular cells is found in the conjugation cartilage, while the underlying tissue keeps its trabecular structure.

This comparison thus shows that the first detectable alteration takes place in the cartilage.

Histological alterations of this type have been observed also in the proximal epiphyses of the humerus, of the fibula at the chondriocostal joint, etc., and finally in the vertebral bodies where, in addition to loss of the trabecular structure, we found deficient ossification and, in its central area, an abnormal prevalence of red marrow. The histological study has also shown that beryllium carbonate has caused a delayed ossification of the patella, relative to the control rats.

Histological examination of the sections of primary adipose tissue shows that, in the rats treated with beryllium carbonate, it usually is in the gland-like form with compact or slightly foamy cells. This finding is in agreement with the strong loss of weight of the test rats.

The early appearance of hyperproliferation of the serial cartilage cells should be emphasized, because of possible histopathogenic significance. The clear prevalence of this tissue indicates the possibility of a primary

disequilibration of the elements of normal morphogenesis with insufficient vascular activity, and insufficient activity of the chondroclasts, of the osteoblasts, etc.

Effect of cod liver oil and of vitamin D. In Experiments 2 and 3, we were obliged to discontinue administration of Riedel beryllium carbonate a few days after beginning the treatment because of the poor condition of the rats. In these intervals, we added to the diet 3-4 drops of cod liver oil, which did not, however, change the final rickets picture. In Experiment No. 5, after a 48-day treatment, the rats were divided in two groups; one of them received daily two drops of Vigantolo, in addition to Landau beryllium carbonate. The rats of this group had an average weight higher than the other group, but the pre-existing rachitic lesions were not modified.

We could thus conclude that the rachitic lesions caused by beryllium carbonate treatment cannot be modified by administration of vitamin D.

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Key:

1. The toxicity of beryllium/glucinium. Thesis
2. Givens and unknowns of alimentary problems
3. Etiopathogenesis of rickets

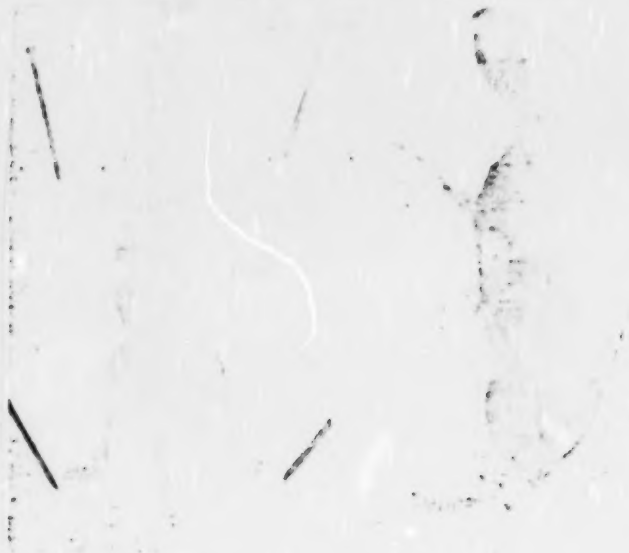

Fig. 1-2. Experiment No. 3. Rat 7A treated with Rieder beryllium carbonate. Decreased bone development. Poor calcification. Thickened epiphyseal cartilage at the head of tibia and of humerus. Strong curving of spine.

Fig. 3-4. Experiment No. 3. Rat 6B, control.

Fig. 5-6. Experment No. 3. Rat 8A treated with Rieder beryllium carbonate. Poorly developed bones. Poor calcification. Irregular development and calcification of the epiphyses of knee joints. Strong curving of spine.

Fig. 7-8. Experiment No. 3. Rat 7B, control.

Fig. 9-10. Experiment No. 5. Rat 1A treated with Landau beryllium carbonate. Sizable increase of tibial epiphyseal cartilage. Decreased calcification of the distal femur epiphysis and proximal humerus epiphysis. Strong curving of spine. Hypocalcification of vertebrae.

Fig. 11-12. Experiment No. 5. Rat 2B, control.

Fig. 13-14. Experiment No. 5. Rat $1A_1$ treated with Landau beryllium carbonate. Reduced bone development. Increased thickness of the conjugation cartilage at the head of tibia and of humerus. Strongly curved spine. Poorly calcified vertebrae.

Fig. 15-16. Experiment No. 5. Rat 5B, control.

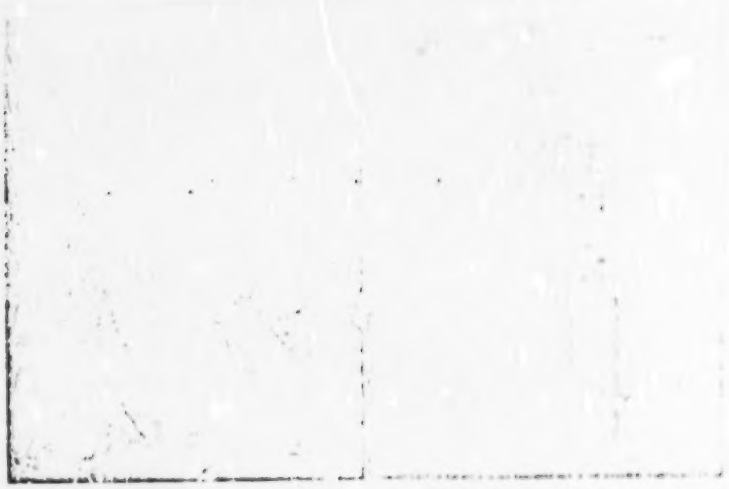

Fig. 17. Experiment No. 2. Rat 4A after 26 days of treatment with Rieder beryllium carbonate. The conjugation cartilage of the head of tibia shows a large increase of globular cells of the serial layer, especially toward the median side. Many cartilage shoots dip in a disorderly fashion into the underlying tissue which has lost its trabecular structure. Scarcity of marrow and vessels.
Fig. 18. Experiment No. 2 Rat 2B , control. Conjugation cartilage of the head of tibia.

Fig. 19. Experiment No. 3. Rat 5A after 14-day treatment with Rieder beryllium carbonate. The conjugation cartilage of the head of tibia is thickened in the serial zone. Long shoots of globular cells enter the underlying tissue which lacks a clear trabecualr structure. The ossification area is rather dense.
Fig. 20. Experiment No. 3. Rat 5B, control. Conjugation cartilage of the head of tibia.

Fig. 21. Experiment No. 3. Rat 5A after 14-day treatment with Rieder beryllium carbonate. At the head of the fibula there is an abnormal thickening of the serial cartilage corresponding to the layer of globular cells. The underlying tissue does not have trabecular structure, but is rather dense and has few marrow gaps. Poor vascularization.
Fig. 22. Experiment No. 3. Rat No. 5B, control. Conjugation cartilage of the head of fibula.

Fig. 23. Experiment No. 3. Rat 5A after 14-day treatment with Rieder beryllium carbonate. Chondrio-costal joint. The increase of globular cells of the serial cartilage is fairly sizeable. Rather dense structure in the underlying ossification zone.
Fig. 24. Experiment No. 3. Rat 5B, control. Chondrio-costal joint.

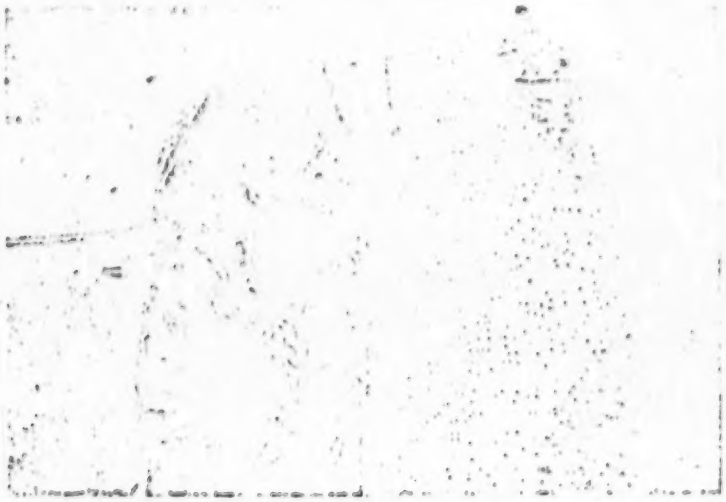

Fig. 25. Experiment No. 5. Rat 1A after 48-day treatment with Landau beryllium carbonate. Strong proliferation of globular cells of the serial layer in the conjugation cartilage of the head of tibia. The shoots dip into the underlying tissue which has lost its trabecular structure and is quite dense, with irregular gaps.
Fig. 26. Experiment No. 5. Rat 2B , control. Conjugation cartilage of the head of tibia.

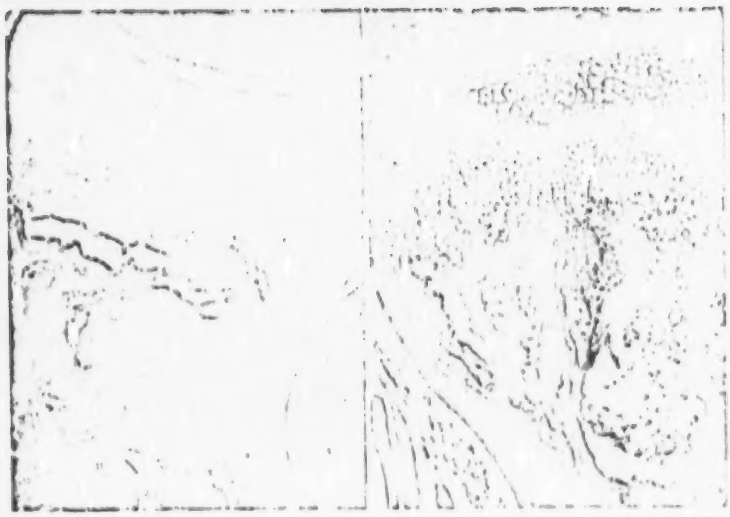

Fig. 27. Experiment No. 5. Rat 1A after 48-day treatment with Landau beryllium carbonate. Vertebra with increase of globular cells, dense structure of the ossification area, marrow hyperplasia in the central part.
Fig. 28. Experiment No. 25. Rat 2B, control. Vertebra.

Fig. 29. Experiment No. 5. Rat 1A after 48-day treatment with Landau beryllium carbonate. Longitudinal section of the vertebral bodies in the area of strongest curvature. Wall deformation, marrow hyperplasia and almost complete disappearance of trabecular structure are clearly visible.